(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 November 2003 (20.11.2003)

PCT

(10) International Publication Number
WO 03/095603 A1

(51) International Patent Classification[7]: C12M 3/06, C12N 5/06

(21) International Application Number: PCT/SG02/00184

(22) International Filing Date: 14 August 2002 (14.08.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
20202359-6 22 April 2002 (22.04.2002) SG

(71) Applicant *(for all designated States except US)*: **CORDLIFE PTE LTD.** [—/SG]; 1 Ordchard Boulevard, #08-08, Camden Medical Centre, Singapore 248649 (SG).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **YU, Hanry** [CN/SG]; Block 603, Choa Chu Kang, St 62#05-45, Singapore 680603 (SG). **BESTED, Soren, Muller** [DK/SG]; 136 Hill View Av #09-04, Singapore 669598 (SG). **FANG, Steven** [SG/SG]; Apt. Blk 187, Pasir Ris, St 11, #13-90, Singapore 510187 (SG). **ANG, Cheng, Eng** [SG/SG]; Blk 111 Bt Batok West, Ave 6#13-132, Singapore 65011 (SG).

(74) Agent: **ELLA CHEONG MIRANDAH & SPRUSONS PTE LTD**; 111 North Bridge Road, #22-01, Peninsula Plaza, Singapore 179098 (SG).

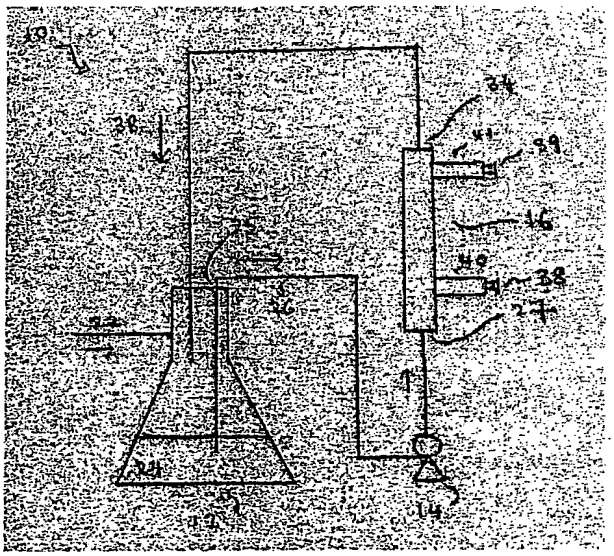
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CELL CULTURE SYSTEM



(57) Abstract: In one aspect of the present invention, there is provided a cell culture system comprising a reservoir, a pump, a hollow fibre cartridge member, the hollow fibre cartridge member further comprising a housing, a number of hollow fibres which are selectively permeable membranes located within said housing and a cell matrix material wherein the cell matrix material is arranged about or within the plurality of hollow fibres in a manner which allows growth of cells and the cell matrix material is selected from the group consisting of collagen, carboxymethylcellulose, chondoitin sulphate, chitosan- polyvinyl pyrrolidone and hyaluronic acid. The present invention also relates in other aspects to a process for isolating cells, a use of a cell culture system and a population of cells and/or a biopharmaceutical product produced by the cell culture system or process of the present invention.

WO 03/095603 A1

CELL CULTURE SYSTEM

Field of the Invention

The present invention relates to a cell culture system with hollow fibre membranes. In particular, the present invention relates to a cell culture system with hollow fibre membranes for culturing cells such as stem cells, and especially those obtained from umbilical cord blood. The present invention also relates to a process for producing a population of expanded cells and/or a biopharmaceutical product. The present invention also relates to a use of a cell culture system and a population of expanded cells and/or a biopharmaceutical product.

Background of the Invention

Cell culture technology has been developed to increase the quantity of cells for use in studies or therapeutic applications. Numerous bioreactor configurations have been developed ranging from simple flat flasks to sophisticated microcarrier and fluidised bed bioreactors.

Normal cell growth is tightly regulated by cues from an extra-cellular matrix which triggers complex intracellular programs. Each cell has contact with a neighbouring cell and the extra cellular matrix. The extra cellular matrix provides cells with a scaffold where the cells are able to be attached. The extra cellular matrix also serves as a dynamic cellular microenvironment which can mediate information to and from the cells and store and protect essential regulatory factors produced by the cells.

The hollow fibre reactor typically mimics a mammalian capillary system of blood vessels. The capillaries which are the smallest vessels in the body connect the arteries and veins in a mesh-like network and are analogous in the hollow fibre reactor to the sheets of hundreds of synthetic hollow fibres. A liquid medium is pumped through these hollow fibres and provides a similar function as the flow of blood through a capillary network. The liquid medium serves to nourish the cells in a hollow fibre reactor and which allows growth of the cells on the outside of the hollow fibres.

Hollow fibre membrane bioreactors have also been used as a simple and economical culture system for anchorage dependent cells which allows continuous flow of the liquid medium in an automated fashion, thereby maintaining a fresh supply of nutrients and allows for removal of metabolic waste products produced by the cells. The typical cells were allowed to anchor onto the hollow fibre membrane either inside or outside the lumen.

However, one disadvantage with the hollow fibre reactors is that cells need to be attached to the membrane for proliferation and function, and such attachment often leads to membrane fowling which thereby leads to deterioration of the mass transfer properties of the hollow fibre membrane. The cells also need to be detached from the membrane when the cells are harvested for re-proliferation or application.

Accordingly, it is a desired object of the present invention to provide a cell culture system based on a hollow fibre membrane bioreactor design for culturing cells without the need to attach the cells to a hollow fibre membrane.

Other desired objects of the present invention include developing a cell culture system which utilises a biocompatible 3-D cell culture scaffold that supports necessary cellular events for development and design of a bioreactor system that enhances cell growth without compromising cellular characteristics.

Other objects and aspects of the present invention will be apparent from the following description of the present invention.

Summary of the Invention

In accordance with a first embodiment of the present invention, there is provided a cell culture system comprising a reservoir, a pump, a hollow fibre cartridge member, the hollow fibre cartridge member further comprising a housing, a number of hollow fibres which are selectively permeable membranes located within said housing and a cell matrix material wherein the cell matrix material is arranged about or within the hollow fibres in a manner which allows growth of cells without adhering to a surface of the hollow fibre membrane.

Preferably, the cell matrix material is selected from the group consisting of collagen, carboxymethylcellulose, chondroitin sulphate, chitosan-polyvinyl pyrrolidone and hyaluronic acid.

In accordance with a second embodiment of the present invention, there is provided a process for producing a population of expanded cells and/or a biopharmaceutical product comprising the steps of :

- inserting a suspension of cells and a cell matrix material into a hollow fibre cartridge member of a cell culture system as described in the first embodiment of the present invention,
- incubating the suspension of cells and the cell matrix material in the hollow fibre cartridge member under conditions suitable to promote cell growth, and

- removing and harvesting the population of expanded cells once they have reached a desired cell density in the hollow fibre cartridge member.

In accordance with a third embodiment of the present invention, there is provided a population of expanded cells and/or a biopharmaceutical product produced by cells wherein the cells were produced by the cell culture system of the first embodiment of the present invention or by the process of the second embodiment of the present invention.

In accordance with a fourth embodiment of the present invention, there is provided a use of a cell culture system as described in the first embodiment of the present invention in the preparation of a population of cells and/or a biopharmaceutical product.

In accordance with a fifth embodiment of the present invention, there is provided a cell reactor comprising a hollow fibre cartridge member, the hollow fibre cartridge member further comprising a housing, a number of hollow fibres which are selectively permeable membranes located within said housing and a cell matrix material wherein the cell matrix material is arranged about or within the hollow fibres in a manner which allows growth of cells without adhering to a surface of the hollow fibre membrane.

Description of the Invention

The cell culture system preferably also comprises a means for removing and harvesting the cells after the cells have reached a desired number in the hollow fibre cartridge member. Preferably, the means for removing and harvesting the cells is an outlet port which extends from the housing of the hollow fibre cartridge member. Still preferably, the outlet port extends in a transverse direction to the longitudinal axis of the hollow fibre cartridge member. A preferred method of removing and harvesting the cells once they have reached a desired number is to evacuate the cells together with the cell matrix material through the outlet port which extends from a side of the housing of the hollow cartridge member. An inlet port which also preferably extends from the housing in a transverse direction to the longitudinal axis of the hollow cartridge member may also be used to assist in the harvesting and removal of cells from the cell culture system. Typically, whilst the cells and cell matrix material are being evacuated through the outlet port, a displacement fluid such as typically a fresh suspension of cells and cell matrix material, air or an inert gas may be injected through the inlet at the same time as the cells and cell matrix material are being evacuated through the outlet port.

The cell matrix material in the cell culture system is preferably porous and is preferably collagen. Preferably, the cell matrix material is collagen gel and still preferably the cell matrix material is a modified collagen gel.

The cell matrix material may be located either inside the lumen of the hollow fibre or outside the lumen of the hollow fibre but within the hollow fibre cartridge housing. Alternatively, the intracapillary spaces of the hollow fibre may be filled with nutrient medium whilst filling the extracapillary spaces with cells and the cell matrix material.

Preferably, the cell matrix material is applied to an extracapillary space of the hollow fibre cartridge. The extracapillary space is located between the outer surface of a lumen of the hollow fibre and the housing of the hollow fibre cartridge member. Still preferably, the cell matrix material is mixed together with the cells in a suitable medium to form a suspension.

Still preferably, the cell matrix material is collagen and more preferably a collagen gel. A preferred method for applying the collagen to the extracapillary space of the hollow fibre membrane is to firstly precipitate the collagen with acetone from a solution of collagen after which the collagen is dissolved in hydrochloric acid and methanol. Typically, the collagen dissolved in the methanol and hydrochloric acid solution is then preferably stirred for a suitable period of time after which it is then dialysed against distilled water followed by freeze-drying. The lyophilised collagen is then typically dissolved in a phosphate-buffered saline and mixed with a cell suspension to form a collagen/cell suspension mixture. The collagen/cell suspension mixture is then preferably injected into an extra-capillary space of the hollow fibre membrane of the cell culture system typically by way of an inlet port located on the side of the housing and left in the extra-capillary space of the housing.

Still preferably, the cell matrix material is a semi-gel like collagen. The semi-gel like collagen is a partially polymerised collagen product where the extent of polymerisation of collagen is dependent upon the concentration of collagen used and the side chain modification. Typically, the cells are grown in a minimal concentration of collagen (about 0.7 to about 1.5mg/ml) which is sufficient to support the cell growth, proliferation and cell functions and which is significantly lower than the typically higher concentrations of matrix materials (1.5 to 5 mg/ml of natural collagen which gels at 37°C) normally used to entrap cells in other 3D culture configurations. The cell matrix material typically should not completely gel in the hollow fibre membrane so as to hold the cells in place.

Further, the semi-gel like collagen may be modified to have a net positive charge. At this particular combination, the collagen does not polymerise enough to gel, but is sufficient to support cell growth in a three dimensional manner and minimises the need

for anchorage dependent cells to have to attach to the hollow fibre membrane, and hence the need to subject the cells to harsh treatments for subsequent cell detachment.

The polymerisation of collagen for the present invention is typically controlled by chemical modification of the collagen as it is being synthesised. The collagen is typically modified by the removal of either the negative or the positive charge from the collagen chains. Still preferably, cationic collagen is obtained through the modification of the carboxyl group by esterification with low molecular weight alcohol.

The cell matrix material may also be selected from the group consisting of carboxymethylcellulose, chondroitin sulphate, chitosan-polyvinyl pyrrolidone and hyaluronic acid which acts in a similar manner to that described for the embodiment for collagen.

Whilst the cell matrix material may be added to the extra-capillary space as described in embodiments above, the cell matrix material may also be applied to the intra-capillary space in an analogous manner.

The pumps used in this invention are typically peristaltic pumps and may be used alone or together with standard commercially available oxygenators.

The reservoir used in this invention can be any standard commercially available culture media bottle known to the skilled person.

Typically, the hollow fibre membranes are made of a suitable material known to the skilled person. Preferably, the hollow fibre membrane is made from a wide variety of materials, including polymers, graphite, ceramics (including porous glass fibres). Preferably, the hollow fibre material is made from a polymeric material which provides an optimal combination of such properties as tensile strength, melt temperature, and glass transition temperature. Suitable polymeric materials from which hollow fibres can be formed include polypropylene, cellulose (including regenerated cellulose) and cellulose acetate), polyethylene, polysulfone, polymethyl methacrylate, polyacrylonitrile, poly(vinylidene fluoride) and similar materials whose properties make them suitable for this invention.

Preferably, the hollow fibre has a pore size of about 0.25 to 0.75μm, still preferably 0.3 to 0.6μm, more preferably 0.4 to 0.6μm and still preferably 0.5μm. which permit the passage of suitable cellular products and media components. Preferably, the optimal diameter of the hollow fibre should be less than 200μm, more preferably less than 180μm and still more preferably less than 150μm so as to allow sufficient diffusion of oxygen.

Typically, the housing which encloses the number of hollow fibres is cylindrical in shape. The housing preferably encloses a plurality of hollow fibres which are selectively permeable membranes.

Preferably, the process of the second embodiment comprises a further step of assaying for cell growth.

Preferably, the step of inserting a suspension of cells and a cell matrix material into a hollow fibre cartridge member of the process of the second embodiment is by way of injecting the suspension of cells and the cell matrix material into an extracapillary space of the hollow fibre.

Preferably, the process of the second embodiment is conducted with the cell matrix material being collagen, more preferably a collagen gel and still more preferably a partially polymerised collagen gel.

Preferably, the process of the second embodiment of the present invention comprises a further step of reinjecting a fresh suspension of cells and a cell matrix material once the population of expanded cells has been removed and harvested.

Brief Description of the Drawings

The present invention will now be illustrated by describing a small number of embodiments, by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 is a schematic view of a cell culture system in accordance with an embodiment of the present invention;

Fig. 2 is an expanded plan view of a hollow fibre cartridge used in the cell culture system as shown in Fig.1.; and

Fig. 3 is a schematic flow diagram of a process for producing a population of cells in accordance with another embodiment of the present invention.

Description of Preferred Embodiment(s)

The following description of the preferred embodiment(s) of the present invention provides one or more examples of the present invention and serves to illustrate the present invention and should not be construed as limiting the generality and scope of the claims.

In the accompanying figures, there is shown in Fig.1 a cell culture system **10** including a reservoir **12**, a pump **14**, and a hollow fibre cartridge member **16**. The reservoir **12** has an inlet port **22**, which is located towards an upper end of the reservoir **12**. The inlet port **22** allows introduction of a nutrient fluid **24** into the reservoir **12** so that

the nutrient fluid **24** is stored in the reservoir **12** both in operation and in storage of the cell culture system **10**.

The reservoir **12** is in fluid communication with the pump **14** by way of a line **26**, which exits the reservoir **12** by way of an outlet **25**. The line **26** may be any type of fluid conduit such as a hose, an enclosed tube and the like. The pump **14** when in operation pumps the nutrient fluid **24** around the cell culture system **10** by way of the line **26** as will be explained in greater detail below.

The pump **14** is also in fluid communication with an inlet **27** of the hollow fibre cartridge member **16** such that the nutrient fluid **24** is pumped from the outlet **25** of the reservoir **12** to the inlet **27** of the hollow cartridge member **16**.

The detailed features of the hollow fibre cartridge member **16** are best seen in Fig.2 where there is shown an expanded plan view of the hollow fibre cartridge member **16** having a housing **28**. The hollow fibre cartridge member **16** further comprises within the housing **28**, a plurality of hollow fibres **17** which extend along the length of the hollow cartridge member **16**.

In order to best illustrate the invention, there is only shown one hollow fibre **17** in Fig.2 but it should be understood that this embodiment of the present invention has a plurality of hollow fibres within the housing **28** of the hollow fibre cartridge member **16**. The plurality of hollow fibres **17** are semi-permeable membranes which form a bundle of hollow fibres within the housing **28**. An intracapillary space **19** is formed within an inside surface of a lumen of the hollow fibre **17**. An extracapillary space **21** is also formed between an outside surface of the lumen of the plurality of hollow fibres **17** and the housing **28**.

The hollow fibre cartridge member **16** receives the nutrient fluid **24** from the reservoir **12** and the nutrient fluid **24** is pumped into the intracapillary space **19**. The cells to be cultured are injected in the extracapillary space **21** of the plurality of hollow fibres **17** together with collagen as will be described below. The nutrient solution **24** permeates from the intracapillary space **19** of the hollow fibre membrane **17** into the extracapillary space **21**. The waste products of the cells permeate from the extracapillary space **21** to the intracapillary space **19** of the hollow fibre membrane **17** and exit the hollow fibre membrane by way of outlet **34**. The cells are mixed with collagen in a suspension in a first syringe **38** and the suspension is injected into an inlet port **40** which extends from the housing **28** in a transverse direction to a longitudinal axis of the hollow cartridge member **16**. The cells will be cultured in the extra-capillary space **21** of the hollow fibre **17**. The

cultured cells are harvested by a withdrawal of the cultured cells and collagen through an outlet port **41** which is also connected to a second syringe **39**. The removal and harvesting of the cultured cells and the collagen through the outlet port **41** into the second syringe **39** occurs simultaneously with the fresh injection of cells and collagen from the first syringe **38** through the inlet port **40** to the extracapillary space **21** of the hollow fibre cartridge member **16**.

The preferred method for applying the collagen to the extracapillary space **21** of the hollow fibre membrane **17** is to firstly precipitate from a stock solution of 3mg/ml of collagen in 0.012M HCl (Vitogen 100 from Collagen Corp.) with 400ml acetone after which the collagen is dissolved in 200ml 0.1MHCl (hydrochloric acid) containing methanol, stirred at 4°C for 6 days and then dialysed against distilled water for an additional 4 days at 4°C followed by freeze-drying. The 0.1M HCl is obtained by diluting concentrated HCl (12M) with a suitable amount of methanol. In this example, 1.6ml of conc. HCl is diluted with 198.2 ml of methanol. The lyophilised collagen is then dissolved in a phosphate-buffered saline and mixed with a cell suspension to form a collagen/cell suspension mixture. The collagen is dissolved at a concentration of 1.5mg/ml and placed in the first syringe **38**. The collagen/cell suspension mixture is then injected from the first syringe **38** into the extra-capillary space **21** of the hollow fibre membrane **17** of the cell culture system **10** typically through the inlet port **40** located on the side of the housing **28**.

The cell matrix material in this embodiment is a semi-gel like collagen. The semi-gel like collagen is a partially polymerised collagen product where the extent of polymerisation of collagen is dependent upon the concentration of collagen used and the side chain modification. The cells are grown in a minimal concentration of collagen (0.7 to 1.5mg/ml) which is sufficient to support the cell growth, proliferation and cell functions and which is significantly lower than the typically higher concentrations of matrix materials (1.5 to 5 mg/ml of natural collagen which gels at 37°C) normally used to entrap cells in other 3D culture configurations. The semi-gel like collagen should not completely gel in the hollow fibre membrane so as to hold the cells in place.

The general protocol for cell inoculation in the cell culture system **10** occurs is as follows.

The inlet **27** and outlet **34** of the hollow fibre cartridge member **17** are closed by way of a pair of clamps **30** and **32** respectively. Then, 5×10^5 to 2×10^6 cells/ml is resuspended in a volume of conditioned medium and collagen which is about 1.5 times

the extracapillary space **21** volume of the hollow fibre cartridge member **17**. The cell suspension is drawn into a first syringe **38** which is connected to the inlet port **40** as shown in Fig. 2. An empty second syringe **39** is also connected to the outlet port tubing **41** as also shown in Fig. 2. The cell suspension is then flushed through the extra-capillary space **21** of the hollow fibre **17** at least 4 or 5 times to uniformly and completely fill the extracapillary space **21** with cells and to ensure displacement of all air bubbles from the extracapillary space **21** into an empty syringe. After the cells have been suitably inoculated, the clamps **30** and **32**, respectively, are opened so as to allow the pumping of nutrient fluid **24** through the hollow fibre **17**.

The cells which are injected at an initial density of 5×10^5 to 2×10^6 cells/ml are then incubated in the cell culture system **10** for a period of more than 1 week. After the incubation period has expired, the cells are harvested and a cell count is performed using a haemocytometer.

The general procedure for harvesting of cells is as follows. The inlet **27** and outlet **34** of the hollow fibre cartridge member **17** are closed by way of the clamps **30** and **32** respectively. Then, the cell suspension is gently withdrawn from the extracapillary space **21** into an empty closed syringe **39** while simultaneously pushing air into the extracapillary space **21** with another syringe **38**. The inlet port **40** with the syringe **38** containing the cell suspension is clamped. The cell suspension is transferred into a centrifuge tube (not shown) and subsequent cell count and flow cytometry is performed.

The type of collagen used is dependent on the cell type used. For example, certain cells grow faster in type I collagen whilst other cells grow faster in tin type II collagen. The chemically modified collagen will be mixed with the cells and injected into/outside the lumen of the hollow fibre. The semi-gel like collagen provides the support the cells require for optimal growth and allows the cells to grow in a three dimensional manner.

The extra cellular material used in the preferred embodiment is collagen and a suitable collagen is commercially available as product Vitrogen 100 from Collagen Corp., Palo Alto, California United States of America.

The cells which are used in the preferred embodiment of the present invention are selected from the following 2 cell lines.

The first cell line is Chinese Hamster Ovary (CHO) cells obtained from the National University of Singapore, Bioprocessing Technology Centre, Singapore that express recombinant green fluorescence protein (GFP). CHO cells are anchorage dependent cells.

The second cell line is KG-1a obtained from American Type Culture Collection, ATCC, CCL-246, which are primitive haematopoietic cells which express surface antigens CD34 but not CD38. The cells with a phenotype of CD34 positive and CD38 negative are of particular interest since these are haematopoietic stem cells (HSC) which reside in the bone marrow and are of particular interest in a wide range of applications due to their ability to differentiate into the different cell blood types and are critical in stem cell transplantations.

Both cell lines are grown in the cell culture system **10** as described herein. A flow cytometry system is used to analyse cell numbers and phenotype. GFP expressed by CHO cells will be assayed by using the Flourescein isothiocyanate (FITC) channel. CD34 and CD38 antigens will be assayed with Phycoerythrin (PE) conjugated and FITC conjugated antibodies. Flow cytometry will be performed at regular intervals after the cells are introduced in to the cell culture system **10**.

CHO cells expressing green flourescein protein (GFP) is used as a model to illustrate that the cell culture system 10 of the present invention can be used for producing a population of expanded cells and a recombinant product (in this case GFP). Further, since these cells are anchorage dependent cells, the following experimental procedure will demonstrate that the cell culture system **10** does not require attachment of the cells to a hollow fibre membrane surface.

KG-1a cells are used mainly to illustrate that the cell culture system 10 can be used to expand cells. Assaying the cell surface antigen (ie CD34 and CD38) with flow cytometry is to show that, under such cell culture system conditions, the surface markers are not affected. Please refer to Figure 3 for the steps involved.

In Fig. 3, there is shown a preferred process in accordance with the present invention for increasing the number of KG-1a cells and CHO-GFP cells.

Firstly, the cells are resuspended in conditioned collagen at a concentration of 1.5mg/ml at a concentration of 5×10^5 to 2×10^6 cells/ml at 1.5 times the volume of the extracapillary space **21** of the hollow cartridge member **16**. The cell/collagen suspension is injected by way of a syringe as described herein into the extracapillary space **21** of the hollow cartridge fibre **17** and through the inlet port **40**. The cells are incubated under suitable conditions for more than one week after which the cells are harvested by way of removing the cells in collagen suspension from the outlet port **41**. The cells/collagen suspension is washed with cold phosphate buffered saline. An aliquot of the cells is taken to instigate a cell count on a haemocytometer.

In respect of KG-1a cells, 1 x 10^5 cells is resuspended in 50µl of phosphate buffered saline together with 2% bovine calf serum. 20µl of anti-CD34 FITC conjugated and anti-CD38 PE conjugated antibodies is added and incubated on ice for 1 hour. The suspension is washed twice with cold phosphate buffered saline and resuspended in 500µl of phosphate buffered saline before samples thereof are run in a flow cytometer.

In respect of CHO-GFP cells, a total of 1 x 10^5 cells is resuspended in 500µl of phosphate buffered saline before samples thereof are run in a flow cytometer.

The measurement of specific bands of fluorescence emitted by the cells is achieved by using flow cytometry. The cells are stained with fluorochromes, for example FITC, or PE conjugated antibodies. The measurement of fluorescence provides a measure of the amount of GFP produced by the CHO-GFP cells and in the case of KG-1a cells, the measurement of the expression of CD34 and CD38. The amount of fluorescence emitted by the CHO cells provides information as to whether the expanded number of cells have lost the expression of the recombinant protein i.e. GFP after such culture has been conducted using the cell culture system of the present invention.

Modifications and variations such as would be apparent to a person skilled in the art are deemed to be within the scope of the present invention. It is to be understood that the scope of the claims should not be restricted to the particular embodiments and examples described herein.

CLAIMS

The claims defining the invention are as follows:

1. A cell culture system comprising a reservoir, a pump, a hollow fibre cartridge member, the hollow fibre cartridge member further comprising a housing, a number of hollow fibres which are selectively permeable membranes located within said housing and a cell matrix material wherein the cell matrix material is arranged about or within the plurality of hollow fibres in a manner which allows growth of cells without adhering to a surface of the hollow fibre membrane.

2. A cell culture system according to claim 1, wherein the cell matrix material is selected from the group consisting of collagen, carboxymethylcellulose, chondroitin sulphate, chitosan-polyvinyl pyrrolidone and hyaluronic acid.

3. The cell culture system according to claim 1 or 2, which further comprises a means for removing and harvesting the cells after the cells have reached a desired number in the hollow fibre cartridge member.

4. The cell culture system according to claim 1, 2 or 3, wherein the cell matrix material is collagen gel.

5. The cell culture system according to claim 4 , wherein the collagen gel is a chemically modified collagen gel.

6. The cell culture system according to claim 5, wherein the chemically modified collagen gel is formed by esterification of the collagen gel with a low molecular weight alcohol.

7. The cell culture system according to any one of claims 4 to 6, wherein the collagen gel is partially polymerised gel.

8. A cell culture system according to any one of claims 1 to 7 wherein the cell matrix material is located within an intracapillary space of the hollow cartridge member.

9. A cell culture system according to any one of claims 1 to 7, wherein the cell matrix material is located within an extracapillary space of the hollow cartridge member.

10. A cell culture system according to any one of the preceding claims, wherein the cells are KG-1a cells or CHO-GFP cells.

11. A process for producing a population of expanded cells and/or a biopharmaceutical product comprising the steps of :

- inserting a suspension of cells and a cell matrix material into a hollow fibre cartridge member of a cell culture system as described in any one of claims 1 to 10,

- incubating the suspension of cells and the cell matrix material in the hollow fibre cartridge member under conditions suitable to promote cell growth, and
- removing and harvesting the population of expanded cells once they have exceeded a desired number in the hollow fibre cartridge member.

12. A process for producing a population of expanded cells and/or a biopharmaceutical product according to claim 11, further comprising the step of assaying for cell growth.

13. A process for producing a population of expanded cells and/or a biopharmaceutical product according to claim 11 or 12 wherein the step of inserting a suspension of cells and a cell matrix material into a hollow fibre cartridge member is by way of injecting the suspension of cells and the cell matrix material into an extracapillary space of the hollow fibre.

14. A process for producing a population of expanded cells and/or a biopharmaceutical product according to claim 13, wherein the injecting step is conducted wherein the cell matrix material is collagen.

15. A process for producing a population of expanded cells and/or a biopharmaceutical product according to claim 14, wherein the collagen is a partially polymerised collagen gel.

16. A process for producing a population of expanded cells and/or a biopharmaceutical product according to any one of claims 11 to 15, further comprising the step of reinjecting a fresh suspension of cells and a cell matrix material once the population of expanded cells has been removed and harvested.

17. A process for producing a population of expanded cells and/or a biopharmaceutical product according to any one of claims 11 to 16, wherein the cells are selected from KG-1a cells or CHO-GFP cells.

18. A population of expanded cells and/or a biopharmaceutical product produced by the process of any one of claims 11 to 17.

19. A cell reactor comprising a hollow fibre cartridge member, the hollow fibre cartridge member further comprising a housing, a number of hollow fibres which are selectively permeable membranes located within said housing and a cell matrix material wherein the cell matrix material is arranged about or within the hollow fibres in a manner which allows growth of cells without adhering to a surface of the hollow fibre membrane.

20. The cell reactor according to claim 19, wherein the cell matrix material is selected from the group consisting of collagen, carboxymethylcellulose, chondroitin sulphate, chitosan-polyvinyl pyrrolidone and hyaluronic acid.

21. Use of a cell culture system according to any one of claims 1 to 10 in the preparation of a population of cells and/or a biopharmaceutical product.

22. A population of expanded cells and/or a biopharmaceutical product produced by cells wherein the cells were produced by the cell culture system according to any one of claims 1 to 10. or by the cell reactor of claim 20 or 21.

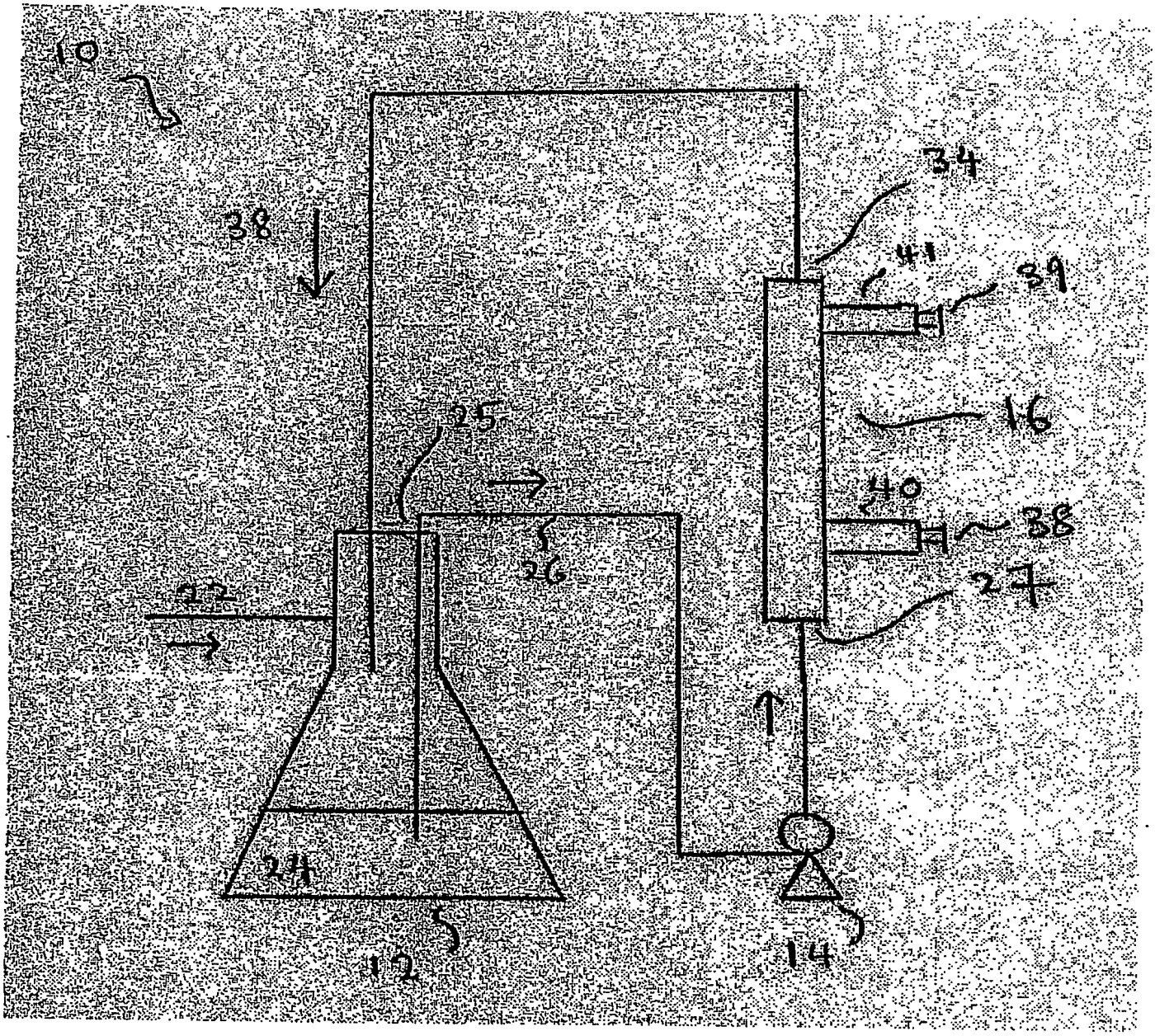


Fig 1.

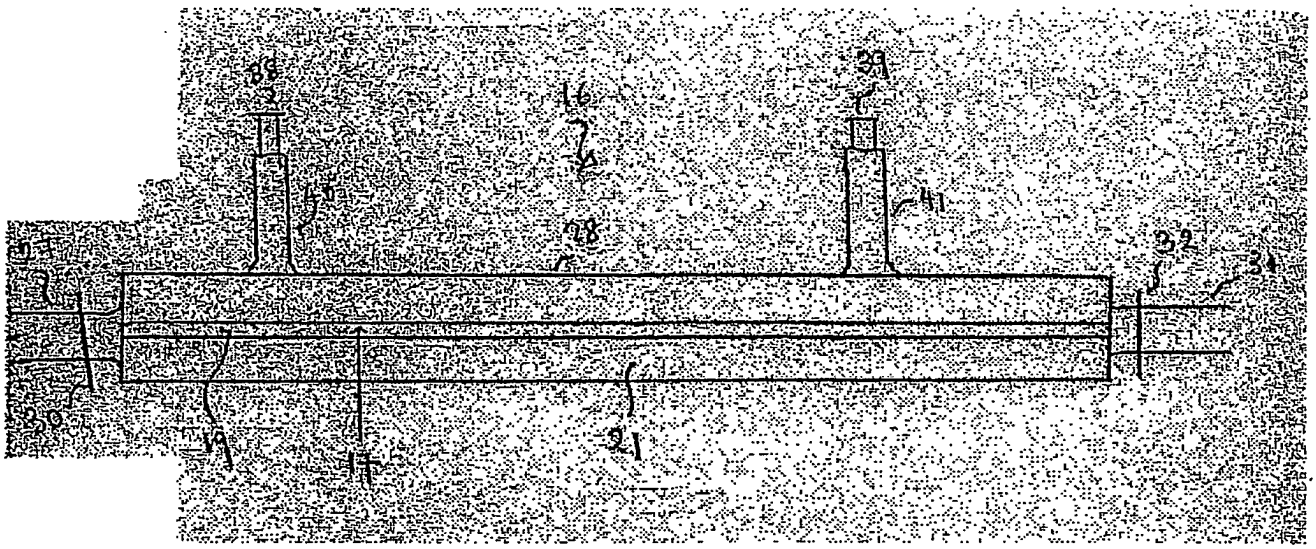


Fig 2.

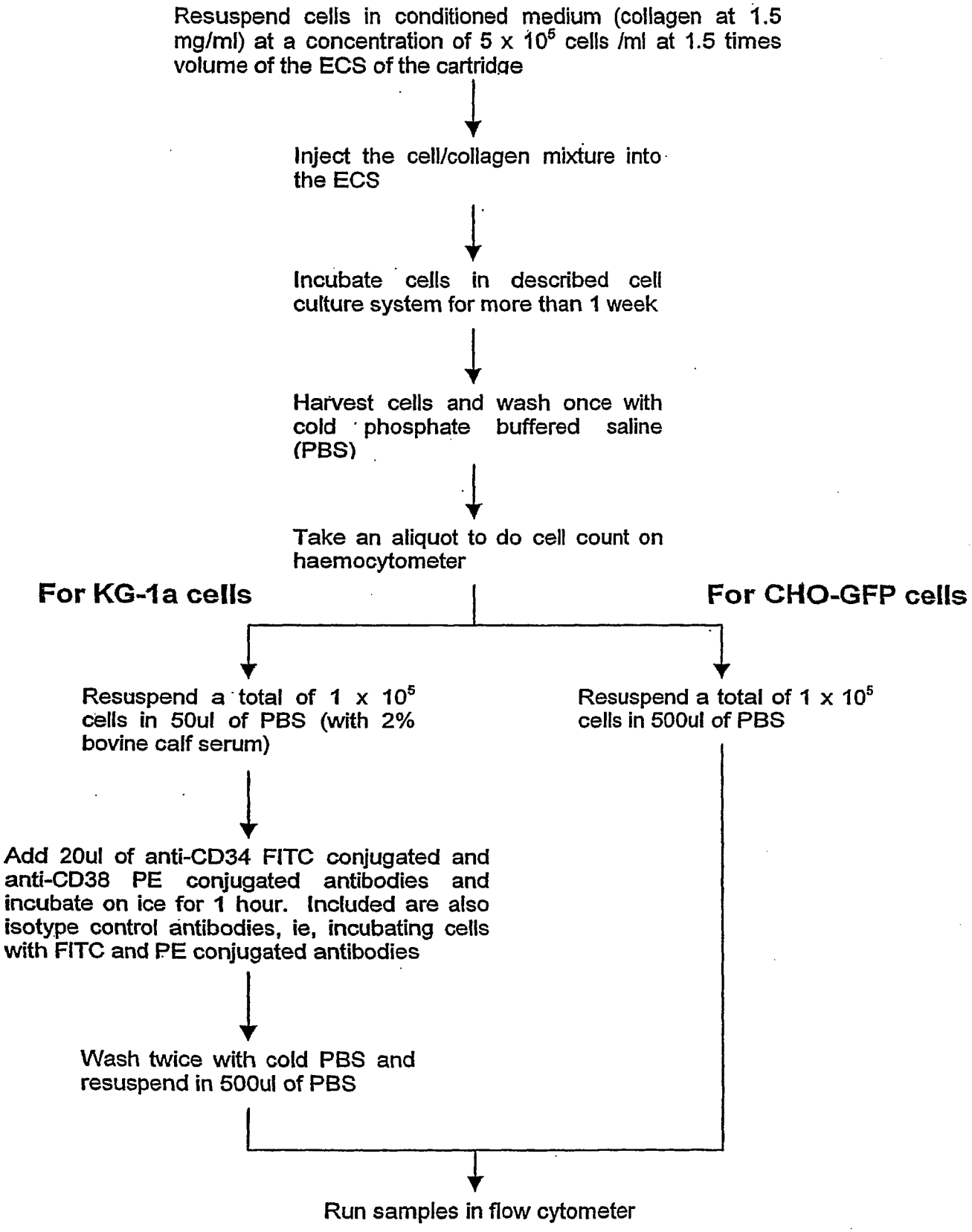

Resuspend cells in conditioned medium (collagen at 1.5 mg/ml) at a concentration of 5 x 10^5 cells /ml at 1.5 times volume of the ECS of the cartridge
Inject the cell/collagen mixture into the ECS
Incubate cells in described cell culture system for more than 1 week
Harvest cells and wash once with cold phosphate buffered saline (PBS)
Take an aliquot to do cell count on haemocytometer
For KG-1a cells
For CHO-GFP cells
Resuspend a total of 1 x 10^5 cells in 50ul of PBS (with 2% bovine calf serum)
Resuspend a total of 1 x 10^5 cells in 500ul of PBS
Add 20ul of anti-CD34 FITC conjugated and anti-CD38 PE conjugated antibodies and incubate on ice for 1 hour. Included are also isotype control antibodies, ie, incubating cells with FITC and PE conjugated antibodies
Wash twice with cold PBS and resuspend in 500ul of PBS
Run samples in flow cytometer

Fig. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 02/00184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12M3/06 C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 605 835 A (CERRA FRANK B ET AL) 25 February 1997 (1997-02-25)	1-5, 8-14, 16-22
Y	abstract figures 4,11,12 column 13, line 30-35 column 15, line 60 -column 16, line 60 column 18, line 35 - line 43 column 23, line 23 - line 36-58; example 7	6,7,15
X	US 5 948 655 A (BADER AUGUSTINUS) 7 September 1999 (1999-09-07)	1,2,4,9, 10,18-22
Y	the whole document	5-7
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 October 2002	21/10/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Dumont, E

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NYBERG S L ET AL: "Bilirubin Conjugation In A Three Compartment Hollow Fiber Bioreactor" ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY, 1990., PROCEEDINGS OF THE TWELFTH ANNUAL INTERNATIONAL CONFERENCE OF THE IEEE PHILADELPHIA, PA, USA 1-4 NOV. 1990, NEW YORK, NY, USA,IEEE, US, 1 November 1990 (1990-11-01), pages 443-444, XP010035613 ISBN: 0-87942-559-8	1,2,4,8, 10,18-22
Y	the whole document	5-7
Y	WO 02 31135 A (CHIA SER MIEN ;UNIV SINGAPORE (SG); INST OF MATERIALS RES AND ENGI) 18 April 2002 (2002-04-18) page 5, last paragraph -page 6, line 9 page 10, line 7 -page 11, line 4 page 15, last paragraph -page 16, line 6	5-7,15
Y	US 4 559 304 A (KASAI SHUNJI ET AL) 17 December 1985 (1985-12-17) the whole document	5,6
A	ROZGA JACEK ET AL: "Isolated hepatocytes in a bioartificial liver: A single group view and experience." BIOTECHNOLOGY AND BIOENGINEERING, vol. 43, no. 7, 1994, pages 645-653, XP001106529 ISSN: 0006-3592 the whole document	1-22

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SG 02/00184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5605835 A	25-02-1997	US 5595909 A	21-01-1997
		US 5981211 A	09-11-1999
		AT 120485 T	15-04-1995
		DE 68921974 D1	04-05-1995
		DE 68921974 T2	03-08-1995
		EP 0380610 A1	08-08-1990
		JP 2835629 B2	14-12-1998
		JP 3505965 T	26-12-1991
		KR 131822 B1	11-04-1998
		WO 8911529 A1	30-11-1989
		AU 9031591 A	26-05-1992
		WO 9207615 A1	14-05-1992
US 5948655 A	07-09-1999	DE 4322746 A1	12-01-1995
		AT 171469 T	15-10-1998
		AU 7457994 A	06-02-1995
		DE 59406982 D1	29-10-1998
		WO 9502037 A1	19-01-1995
		EP 0708823 A1	01-05-1996
WO 0231135 A	18-04-2002	AU 1314302 A	22-04-2002
		WO 0231135 A1	18-04-2002
		US 2002094569 A1	18-07-2002
US 4559304 A	17-12-1985	JP 1472444 C	27-12-1988
		JP 59028472 A	15-02-1984
		JP 63018469 B	19-04-1988
		DE 3382359 D1	05-09-1991
		EP 0101285 A2	22-02-1984